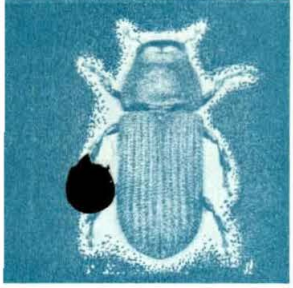
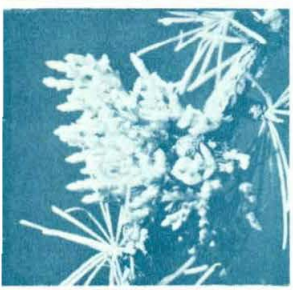

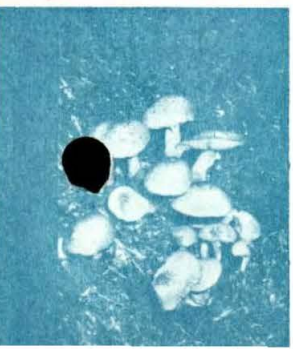

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RESPONSE OF SELECTED SOIL FUNGI TO FUMIGATION AT THE COEUR d'ALENE NURSERY

by

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ABSTRACT

Two soil sampling methods were used to determine numbers and distribution of propagules of *Fusarium* spp. and competitive saprophytes including *Trichoderma* sp., *Penicillium* spp., and *Aspergillus* spp. in fumigated and in nonfumigated nursery soils.

Data indicate that fumigation for root disease control may have been unnecessary in some units, and that effectiveness of fumigation, in terms of reducing *Fusarium* populations, varied from being effective to being ineffective and even stimulatory in one instance.

INTRODUCTION

Fumigation, partially for root disease control, has been an accepted practice in conifer nursery management for nearly 10 years at the National Forest Nursery at Coeur d'Alene, Idaho; yet seedling mortality apparently due to root pathogens has continued to increase. Fumigation is an expensive practice and the question arises: What, in terms of disease control, are we buying with fumigation?

The following preliminary soil evaluations to determine effects of fumigation on selected soil fungi, including both pathogens and saprophytes, were established to shed light on the above question.

METHODS AND MATERIALS

Field

In early September 1974 seedbeds were fumigated at a rate of 350 pounds (157.5 kg) per acre (.4 ha) with a material containing 98 percent methyl bromide and 2 percent chloropicrin. Five seedbeds in widely separated sections were not fumigated.

Six days following fumigation, soil samples, each consisting of five 2.5 cm x 15 cm soil cores obtained from 60 cm square plots, were taken from each of the 5 nonfumigated and 5 immediately adjacent fumigated seedbeds. Samples from each plot were placed in plastic bags and transported to Missoula for laboratory evaluation.

Soil profile plates, 20 cm x 30 cm x 1.5 cm autoclavable polypropylene plastic plates with 0.5 cm diameter x 1 cm deep holes spaced at 2.5 cm intervals both vertically and horizontally (Anderson and Huber, 1965) were installed in each of the 5 fumigated and 5 adjacent nonfumigated seedbeds. Holes were filled with potato dextrose agar and then covered with black plastic electricians tape according to methods presented by Anderson and Huber (1965). Plates were wrapped in aluminum foil and refrigerated until used 3 to 5 days later.

Once in the field, paired soil pits, one in fumigated and one in immediately adjacent nonfumigated soil, were carefully prepared with a flat shovel vertical to the soil surface and perpendicular to the long axis of each seedbed. Immediately prior to placing profile plates into soil pits, small holes were punched with a sterile needle into the tape directly above the agar-filled holes. Profile plates were placed firmly against the flat surface of soil pits so that the top row of holes was approximately 2.5 cm below ground line and soil was filled in behind the plate to hold it in place. Aluminum foil was placed over the top edge of each plate to exclude soil and water from the agar-filled holes.

After 7 days, plates were removed, placed individually into plastic bags, and taken to Missoula for laboratory study.

Laboratory

Each soil sample was thoroughly mixed, 5 gm to 10 gm portions placed into sterile petri dishes and air dried overnight. Soil dilutions of 1:50 were made with 0.5 percent water agar and 1 ml per plate of the soil dilution plated onto each of three different agar media,

two selective for *Fusarium* species (Edmonds and Heather 1973; Russell [1/]) and one for *Pythium* species (Russell, mimeographed information). Five replications were made for each soil sample on each medium. Generally, petri plates were examined after 2 days, 5 days, and 8 days incubation at 25°C in the dark.

Colonies of *Fusarium* spp., and competitive saprophytes including *Trichoderma* sp., *Penicillium* spp., and *Aspergillus* spp., were counted on all plates. Individual colonies were interpreted as functionally being a single propagule i.e., any structural portion of an organism capable of propagating or reproducing that organism.

Agar "plugs" from holes in soil profile plates were aseptically transferred to petri dishes containing potato dextrose agar and were incubated at 25°C for 3 to 5 days or until fungi growing from plugs could be identified.

Fumigation effects of *Fusarium* populations were tested by subjecting soil dilution plate data to paired t test analyses (Freese, 1967). Confidence level in all cases was P=.05. Other data were not statistically analyzed.

RESULTS

Pythium spp. were not identified on soil dilution plates containing any of the three types of media, however, *Fusarium* spp. and competitive saprophytes were found on all three (Table 1).

Approximately 400 *Fusarium* propagules per gm soil were found in nonfumigated areas in units 4B-24, 5B-18, and 6C-42 (Table 1). In fumigated portions of units 4B-24 and 5B-18, number of *Fusarium* propagules was significantly lower than in nonfumigated portions. However, in unit 6C-42 over 10 times more *Fusarium* propagules were isolated from fumigated than from nonfumigated soils. Soil dilutions for a second set of soil samples from unit 6C-42 yielded similar data.

Small numbers of *Fusarium* propagules were found in both fumigated and nonfumigated beds of units 3A-12 and 3A-3. Fumigation reduced the *Fusarium* population in unit 3A-12.

1/ Personal communication with Ken Russell, State of Washington, Department of Natural Resources, Division Forest Land Management, Olympia Washington.

Table 1.--Soil dilution fungal assays of fumigated and nonfumigated nursery beds.

Unit	Fumigated		Nonfumigated	
	Fusarium spp.	Competitive saprophytes[a]	*Fusarium* spp.	Competitive saprophytes
3A-12	0	10,500[b]	20[c]	325
3A-3	123	12,500	35	2,245
4B-24	0	1,480	360[c]	6,445
5B-18	70	12,500	420[c]	6,250
6C-42	4,440[c]	40	400	250

a *Trichoderma, Penicillium, Aspergillus*

b All values are averaged propagule counts for 15 soil dilution plates (5 for each medium) and are expressed in propagules per gm soil.

c Significantly different (P=.05) from corresponding fumigated or nonfumigated sample.

Soil profile plate data (Table 2) tend to support dilution plate data (Table 1). Soil profile data show that *Fusarium* propagules were infrequently isolated from nonfumigated soils of units 3A-3 and 3A-12 and were generally isolated from nonfumigated soils in units 4B-24, 5B-18, and 6C-42. Frequency of isolation was greatest in unit 6C-42. In fumigated soils, soil profile data show that *Fusarium* spp. were: (1) not isolated from unit 3A-3 and only twice from unit 3A-12; (2) infrequently isolated except at the lowest levels of the soil profile in unit 4B-24; (3) frequently isolated from the uppermost soil levels in unit 5B-18; and (4) frequently isolated from throughout the soil profile in unit 6C-42. Frequency of *Fusarium* spp. isolation was higher in fumigated than in nonfumigated soils throughout the soil profile in unit 6C-42 and at upper soil levels in unit 5B-18.

Soil dilution data show that larger numbers of competitive saprophytes were found in fumigated than in nonfumigated beds of units 3A-12, 3A-3, and 5B-18. In units 4B-24 and 6C-42 the reverse was true. Soil profile data (Table 2) supports soil dilution data (Table 1) except that in unit 5B-18 competitive saprophytes were more frequently isolated from nonfumigated than from fumigated soils.

Table 2.--Comparison of fungi isolated by the plate profile method from fumigated and nonfumigated nursery soils.

Unit	Plate row[b]	Fumigated		Nonfumigated	
		Fusarium spp.	Competitive saprophytes[a]	*Fusarium* spp.	Competitive saprophytes
			[c]		
3A-3	1		8	1	3
	2		6		2
	3		9		6
	4		8		3
	5		7		1
	6		7	1	5
	7		6		2
	8		3		3
3A-12	1		8	1	4
	2		8		2
	3	2	4	1	3
	4		5		3
	5		6	2	3
	6		4		4
	7		7		2
	8		2		3
4B-24	1				
	2		4	4	7
	3		4	2	4
	4		4	3	7
	5			4	7
	6		2	2	2
	7	3	2	3	2
	8	1	4		
5B-18	1	6	1		5
	2	8	1	1	8
	3	4	3	3	6
	4	1	2	4	5
	5	1	3	2	5
	6		5	4	5
	7		4	1	3
	8		2		6
6C-42	1	7	3	6	
	2	7		6	2
	3	9		8	1
	4	8	1	6	
	5	8		6	1
	6	5	3	4	1
	7	7	1	1	3
	8	3		4	5

a *Trichoderma* sp., *Penicillium* spp., and *Aspergillus* spp.

b Plate row number is indicative of closeness to the soil surface, with row 1 being the closest.

c Values are number of times isolated per 11 holes in a given row.

DISCUSSION

It must be recognized that only three different media were used in these evaluations and that selectivity of each influences fungal populations isolated. Time of year also influences fungal populations (Toussoun 1975), but these evaluations were intended only to measure the effects of fumigation on soil fungal populations at one point in time, shortly after treatment. Information obtained in these evaluations, while perhaps not strictly comparable with other work throughout the country because of the above limitations, accomplishes the objective of exploring the initial effects of fumigation on soil fungi at the Coeur d'Alene Nursery.

Data collected from nonfumigated beds of units 3A-3 and 3A-12 show that small numbers of *Fusarium* propagules were found. This in turn suggests that fumigation was unnecessary in these beds. Perhaps these soils are inhibitory in some way to *Fusarium* spp. and possibly may be described as *Fusarium* suppressive soils (Toussoun 1975).

In nonfumigated beds of units 4B-24, 5B-18, and 6C-42 relatively large numbers of *Fusarium* propagules were found. Although *Fusarium* levels seem low compared to those in other forest nurseries (Toussoun 1975) necessity of fumigation in these beds cannot be exactly determined because: (1) we do not know the relationship between pathogen populations, the soil environment, and hosts; and, (2) we cannot assess pathogenicity based on isolation data alone, and therefore, cannot determine numbers of potentially pathogenic propagules. However, if we assume that a certain percentage of the *Fusarium* population has the potential to be pathogenic, i.e., cause disease, and that the soil environment required for survival by a pathogen and a non-pathogen are similar, we can assume that populations were significant and fumigation necessary in these three beds.

Soil dilution data show that fumigation was effective in reducing *Fusarium* populations in units 3A-12, 4B-24, and 5B-18, but increased or did not decrease *Fusarium* populations in units 3A-3 and 6C-42. The relatively small increase in *Fusarium* propagules in unit 3A-3 may reflect sampling error, but the 10-fold increase in unit 6C-42 is significant and supported by soil profile data. Reasons for the increased *Fusarium* population cannot be determined, but it is possible that it relates to differential effects of the fumigant on fungal populations in different soil types.

Even though soil dilution data show that *Fusarium* populations were lower in fumigated than in nonfumigated portions of unit 5B-18, it is important to note that soil profile plate data for the same unit show that frequency of isolation of *Fusarium*

spp. from the uppermost soil layers was greater in fumigated than in nonfumigated portions. This suggests that volatilization of the fumigant into upper soil layers in sufficient quantities to reduce *Fusarium* populations was not accomplished possibly because of: (1) inadequate soil temperatures; (2) improper application techniques; or, (3) soil properties not conducive to effective use of the fumigant. Because roots of newly germinated seedlings will have a high probability of contacting *Fusarium* propagules while growing through this layer, it can be considered that fumigation was ineffective in unit 5B-18.

Soil profile data indicate that *Fusarium* spp. were not totally removed from treated soils in unit 4B-24 as indicated by soil dilution data. In this case, however, the residual population was located at the lowest depths sampled, and is probably not critical since seedling roots will have had time to form mycorrhizal associations before reaching this layer. These latter two situations illustrate the need for employing more than one system when examining soil fungus populations.

The often greater number of competitive saprophyte propagules found in treated than in untreated beds reflects fungal activity stimulated directly by fumigation or indirectly by the differential selectivity of the fumigant in eliminating soil microorganisms competing against the three genera sampled. The reduction in numbers of competitive saprophytes in treated portions of units 4B-24 and 6C-42 may result from differential susceptibility of fungi or from differential fumigant activity in the different soil systems.

CONCLUSIONS

While the data base for these evaluations is small, it does show that:

1. Fumigation may have been unnecessary in some units due to low *Fusarium* populations.

2. Effectiveness of fumigation, in terms of effects on *Fusarium* populations, varied from positive to negative and may be indicative of differential reaction to the soil's biotic and abiotic fractions to fumigants and/or may point to inadequacies in method of application.

3. More than one system for sampling populations of soil microfungi is necessary.

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The key to explaining information obtained in these evaluations and applying it to disease management seems to lie in solving the relationships between biotic and abiotic soil factors, and disease severity. Some information concerning these relationships is available; however, to be applied to our nursery situation, abiotic and biotic soil factors must first be identified. This work is currently under way.

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